states. By our new method, 'cryo-positive stain EM', the microtubule-bound stalks, as well as the ring-like heads and curved tails, were clearly observed. In the no nucleotide state (with apyrase; the post-powerstroke state), the majority of dynein images showed two rings superimposed, suggesting close association of the two heads. When ATP and vanadate were added (the ADP•Vi, pre-powerstroke state), one of the heads moved with respect to the other. There was no detectable difference in the orientation of the stalks between the two nucleotide states; the stalks always pointed at the same angle towards the minus end of the microtubule to which they were bound (Ueno et al., 2008). The results disagree with models in which the stalk rotates on the microtubule and acts as a lever arm to amplify structural changes.
Rotation of the tail relative to the head would change the distance between the stalk tip and the tail-microtubule attachment point. In fact, some ADP•Vi images clearly showed the tail in a more extended state. Based on these results, we propose a new model, in which dynein pulls a microtubule by shortening the distance between its head/stalk and the tail-microtubule attachment, without rotating the stalk.

Platform AZ: Voltage-gated K Channels-Gating

3229-Plat
Thermodynamic Properties of Ionic Currents in *shaker* K^+ Channel Heterotetramers with Different Stoichiometries of ILT Mutations and/or Quadruple Gating Charge Neutralization
Dominique G. Gagnon, Francisco Bezanilla.
University of Chicago, Chicago, IL, USA.
The concurrent substitutions V369I, I372L, and S376T (ILT) in the S4 segment N-terminal end of voltage-dependent *Shaker* K^+ channels uncouple gating currents from ionic conduction (Smith-Maxwell *et al.*, 1998). This ILT mutation is believed to affect the last concerted step for channel activation. We aimed at determining the effect of temperature on steady-state and kinetics of activation of heterotetramers with different stoichiometries of ILT mutations. In addition, we also studied the single voltage sensor *Shaker* containing the ILT mutation in its voltage sensor. The heterotetrameric channels were encoded by concatemerized cDNA of *Shaker* zH4 Δ(6-46), expressed and studied in *Xenopus* oocytes using cut-open oocyte voltage-clamp under temperature ranging from 5-20 °C. The concatemeric channels $4wt_{ILT}$, $3wt_{ILT}$/wt, $2wt_{ILT}$/2wt, wt_{ILT}/3wt and wt_{ILT}/3mut (mut=R362Q/R365Q/R368N/R371Q) were studied. For all constructs, the amplitude of the ionic currents decreased by about 50-75% and the weighted time constants of activation increased by ten to fifteen fold when going from 19 to 5 °C, in a reversible manner. We also measured the steady-state voltage dependence of the conductance to estimate the entropic change during the final transition. The enthalpic and entropic components estimated from the temperature dependence of kinetics of activation for the different constructs provide essential information about the nature of the conformational changes and interactions between subunits. This information is used to refine the proposed model of independent voltage sensors followed by a concerted opening step.
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3230-Plat
A New Approach to the Structural Investigation of the Voltage-Sensitive Domain of Voltage-Gated Cation Channels as a Function of the Transmembrane Voltage
Jing Liu[1], Venkata Krishnan[1], Joseph Strzalka[2], Andrey Tronin[1], J. Kent Blasie[1].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Advanced Photon Source, Argonne National Laboratory, Argonne, IL, USA.
Two fundamentally different approaches, self-assembly from solution and directed-assembly at the water-gas interface, are effective in the vectorial immobilization of the expressed voltage-sensitive domain of the KvAP channel on a suitably alkylated surface of silicon. The solvation of the immobilized protein was subsequently exchanged to form a phospholipid bilayer by incubating in a phospholipid-detergent solution in the presence of BioBeads. The formation of the protein monolayer and the vectorial-orientation of the protein molecules therein were investigated via interferometric X-ray reflectivity. The electron density profile of the tethered protein monolayer is consistent with the profile computed from the crystal structure, irrespective of the preparation procedure. Formation of lipid bilayer will require confirmation via neutron reflectivity using deuterated phospholipid. This approach enables an investigation of the structure of the VSD itself, as well as within the intact Kv-channel, as a function of the applied transmembrane voltage via a number of time-resolved techniques.

3231-Plat
The Importance of Ion Binding for Potassium Channel Inactivation and Recovery
Albert C. Pan[1], Sudha Chakrapani[1], Vishwanath Jogini[2], Luis G. Cuello[3], Doris M. Cortes[3], Eduardo Perozo[1], Benoit Roux[1].
[1]University of Chicago, Chicago, IL, USA, [2]D. E. Shaw Research, Hyderabad, India, [3]Texas Tech University, Lubbock, TX, USA.
Potassium channels control the flow of ions across cell membranes with gating mechanisms involving conformational changes at the intracellular gate and the selectivity filter. Opening of the intracellular gate via external stimuli (activation) results in a transient period of conduction before the selectivity filter undergoes a conformational change, which constricts the permeation pathway (inactivation). When the applied stimulus is removed and the lower gate closes (deactivation), the filter slowly resets to a conductive conformation (recovery from inactivation). Using the KcsA channel as a prototypical model system to examine these issues, a combination of computer simulation (all-atom free energy and potential of mean force computations as well as transition pathway determination using the string method with swarms-of-trajectories) and experiment (electrophysiology and X-ray crystallography) is used to provide new insight into the microscopic mechanism that underlies inactivation and recovery from inactivation. An ion binding event is revealed as a crucial step in resetting the inactive filter during the recovery from inactivation.

3232-Plat
Charge Reversion to Charge Carrying Positions of S4 in Voltage Gated Shaker K-Channels
Vivian González-Pérez[1,2], Katherine Stack[1], **David Naranjo**[1].
[1]Universidad de Valparaíso and CINV, Valparaíso, Chile, [2]Programa de Doctorado en Ciencias mención Neurociencia, Valparaíso, Chile.
Voltage gated Shaker potassium channels increases open probability (Po) by 20-fold with a ~6 mV depolarization. Such high voltage sensitivity is mostly due to the electrophoretic transmembrane relocation of four arginines residues in each of their four voltage sensing protein domains (VSD). These arginines movement across the electric field make possible channel opening upon membrane depolarization. We tested if the positions occupied by the voltage sensing arginines could carry acidic residues. We mutated three of these positions to aspartate: Arg362, Arg365, and Arg368 on an N-type inactivation removed background Shaker channel. All mutations were introduced with the use of the QuikChange kit and the mutation was verified by sequencing. Heterologous expressed in Xenopus oocytes, all mutants showed levels of expression comparable to that of the native channel. To determine voltage sensitivity of these charge reverting modified channels, we measured the voltage dependency of Po at voltages negative enough to observe only sporadic single channel openings in membrane patches containing hundreds of channels. From the exponential relation between Po and voltage we estimated the effective valence of opening in the range of Po ~10-6. All charge mutants showed an effective valence ~50% of that of the native Shaker. These results together with the comparable level of channel expression in oocytes are consistent with the idea the voltage sensitive positions in S4 are not specific for basic residues.
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3233-Plat
Sequential Electrostatic Interactions between E160 in S2 and Arginines in S4 During Voltage Dependent Activation of Kv7.1 Channels
Dick Wu, Kelli Delaloye, Mark Zaydman, Jianmin Cui.
Washington University, Saint Louis, MO, USA.
The fourth transmembrane segment of Kv channels, S4, contains a series of positively charged residues that imparts voltage sensitivity to the channel. Because the insertion of a highly charged peptide into a hydrophobic lipid environment is energetically unfavorable, electrostatic interactions with countercharges in the protein and phospholipids are required to lower this energy barrier. However, once the protein has been inserted into the membrane, what further role do these interactions play? In functional channels, electrostatic interactions are assumed to stabilize voltage sensor movement from a resting to an activated conformation. Although this assumption is at the crux of many models of voltage dependent gating, experimental evidence specifically examining these interactions in functional channels is incomplete. Here, we demonstrate in Kv7.1 channels that the first glutamate in S2, E160 (E1), form state dependent electrostatic interactions with arginines in S4. We used charged MTS reagents to directly probe the environment around E1 after mutating E1 to cysteine. We found that $MTSES^-$ but not $MTSET^+$ modifies E1C, suggesting a positively charged environment around E1. Mutations neutralizing or reversing the charge of the first or fourth arginine in S4 (R1 or R4) change the polarity of the environment around E1C such that $MTSET^+$ modifies E1C in the